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1: J Eukaryot Microbiol. 1999 Nov-Dec;46(6):557-65. Related Articles, Links

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Heterologous expression of a *Trypanosoma cruzi* surface glycoprotein (gp82) in mammalian cells indicates the existence of different signal sequence requirements and processing.

Ramirez MI, Boscardin SB, Han SW, Paranhos-Baccala G, Yoshida N, Kelly JM, Mortara RA, Da Silveira JF.

Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, Escola Paulista de Medicina, Brazil.

Related Resources

Metacyclic trypomastigotes of *Trypanosoma cruzi* express a developmentally regulated 82 kDa surface glycoprotein (gp82) that has been implicated in the mammalian cell invasion. When the non-infective epimastigote stage of the parasite was transfected with a vector containing the gp82 gene, an 82 kDa surface glycoprotein, which was indistinguishable from the metacyclic stage protein, was expressed. In contrast, when the same gene was expressed in transfected mammalian cells, although a large amount of protein was produced, it was not imported into the endoplasmic reticulum and glycosylated. This blockage in targeting and processing could be partially compensated for by the addition of a virus haemagglutinin signal peptide to the amino terminus of gp82. Thus, the requirements for membrane protein processing are distinct in mammals and *T. cruzi*, and an intrinsic feature of the gp82 prevents subsequent sorting to the mammalian cell surface. These results could be useful in the development of new DNA vaccines against *T. cruzi* employing parasite genes encoding immunodominant surface glycoproteins.

PMID: 10568029 [PubMed - indexed for MEDLINE]

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1: Exp Parasitol. 2002 May;101(1):64-8.

Related Articles, Links



Plasmodium falciparum: glycosylation status of Plasmodium falciparum circumsporozoite protein expressed in the baculovirus system.

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Kedees MH, Azzouz N, Gerold P, Shams-Eldin H, Iqbal J, Eckert V, Schwarz RT.

Institut fur Virologie, Medizinisches Zentrum fur Hygiene und Medizinische Mikrobiologie, Philipps-Universitat Marburg, Robert-Koch-Strasse 17, 35037 Marburg, Germany.

Related Resources

We expressed the main surface antigen of *Plasmodium falciparum* sporozoites, the circumsporozoite protein (CSP), in High Five (Trichoplusia ni) insect cells using the baculovirus system. Significant amounts of the recombinant protein could be obtained, as judged by SDS-PAGE, Western blot, and immunofluorescence analysis. The cellular localization for recombinant CSP was determined by immunofluorescence. The high fluorescence signal of the permeabilized cells, relative to that of fixed nonpermeabilized cells, revealed a clear intracellular localization of this surface antigen. Analysis of possible posttranslational modifications of CSP showed that this recombinant protein is only N-glycosylated in the baculovirus system. Although DNA-sequence analysis revealed a GPI-cleavage/attachment site, no GPI anchor could be demonstrated. These analyses show that the glycosylation status of this recombinant protein may not reflect its native form in *P. falciparum*. The impact of these findings on vaccine development will be discussed.

PMID: 12243739 [PubMed - indexed for MEDLINE]

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Glycosylation of the *Enterobacter cloacae* outer membrane protein OmpX in eukaryotic cells

Gijs de Kort, Marcel M. M. Salimans, Paola van der Bent-Klootwijk, Cobie van Heest, Mario J. A. W. M. van Bussel and Jos A. M. van de Klundert

Department of Medical Microbiology, Leiden University Medical Centre, P.O. Box 9600, 2300 RC Leiden, The Netherlands

Received 8 June 1999; revised 23 June 1999; accepted 24 June 1999. Available online 4 August 1999.

Abstract

The topological model of the *Enterobacter cloacae* outer membrane protein OmpX showed three putative glycosylation sites. When OmpX was expressed in bacteria that were cultured under aerated conditions, no glycosylation was observed. The coupling of carbohydrate chains to the *ompX* gene product was also investigated in the eukaryotic baculovirus expression system. For this purpose, a recombinant *ompX* gene-containing baculovirus was made.

Infection of insect cells with this recombinant virus resulted in the production of sufficient amounts of OmpX to study glycosylation. In this system, all potential *N*-glycosylation sites of OmpX were utilized. Furthermore, it became clear that glycosylated OmpX was retained in the insect cells and was not secreted in the medium. Given the fact that OmpX plays a role in the invasion of *E. cloacae* in rabbit enterocytes, glycosylation of this protein occurring only under specific conditions may be involved in this process.

Author Keywords: OmpX; Glycosylation; Virulence



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1: Curr Opin Biotechnol. 1991 Oct;2(5):730-4.

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Mammalian cell gene expression: protein glycosylation.

PubMed Services

Parekh RB.

Oxford GlycoSystems Ltd, Abingdon, UK.

Considerable advances have been made in identifying the factors determining the glycosylation pattern of glycoproteins secreted by mammalian cells. This has allowed a greater appreciation of the way in which recombinant proteins may be glycosylated after expression in a heterologous system. The studies reviewed herein extend the wider view that glycosylation of native and recombinant proteins is a complex event dependent on the protein moiety, the host cell, and also the environment in which transfected cells are cultured. The details of the way in which these factors combine to establish the glycosylation pattern of a secreted protein are now beginning to be unravelled.

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1: Mol Microbiol. 1993 Dec;10(5):1013-28.

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Pilus-facilitated adherence of *Neisseria meningitidis* to human epithelial and endothelial cells: modulation of adherence phenotype occurs concurrently with changes in primary amino acid sequence and the glycosylation status of pilin.

Virji M, Saunders JR, Sims G, Makepeace K, Maskell D, Ferguson DJ.

Department of Paediatrics, University of Oxford, John Radcliffe Hospital, UK.

Adherence of capsulate *Neisseria meningitidis* to endothelial and epithelial cells is facilitated in variants that express pili. Whereas pilated variants of *N. meningitidis* strain C311 adhered to endothelial cells in large numbers (> 150 bacteria/cell), derivatives containing specific mutations that disrupt pilE encoding the pilin subunit were both non-piliated and failed to adhere to endothelial cells (< 1 bacterium/cell). In addition, meningococcal pili recognized human endothelial and epithelial cells but not cells originating from other animals. Variants of strain C311 were obtained that expressed pilins of reduced apparent M(r) and exhibited a marked increase in adherence to epithelial cells. Structural analysis of pilins from two hyper-adherent variants and the parent strain were carried out by DNA sequencing of their pilE genes. Deduced molecular weights of pilins were considerably lower compared with their apparent M(r) values on SDS-PAGE. Hyper-adherent pilins shared unique changes in sequence including substitution of Asn-113 for Asp-113 and changes from Asn-Asp-Thr-Asp to Thr-Asp-Ala-Lys at residues 127-130 in mature pilin. Asn residues 113 and 127 of 'parental' pilin both form part of the typical eukaryotic N-glycosylation motif Asn-X-Ser/Thr and could potentially be glycosylated post-translationally. The presence of carbohydrate on pilin was demonstrated and when pilins were deglycosylated, their migration on SDS-PAGE increased, supporting the notion that variable glycosylation accounts for discrepancies in apparent and deduced molecular weights. Functionally distinct pilins produced by two fully pilated variants of a second strain (MC58) differed only in that the putative glycosylation motif Asn-60-Asn-61-Thr-62 in an adherent variant was replaced with Asp-60-Asn-61-Ser-62 in a non-adherent variant. Fully adherent backswitchers obtained from the non-adherent variant always regained Asn-60 but retained Ser-62. We propose, therefore, that functional



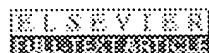
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1: Mol Biochem Parasitol. 1999 Nov 30;104(2):171-83. Related Articles, Links



CL 757. m 655

Analysis of recombinant merozoite surface protein-1 of *Plasmodium falciparum* expressed in mammalian cells.

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Burghaus PA, Gerold P, Pan W, Schwarz RT, Lingelbach K, Bujard H.

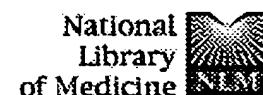
Zentrum fur Molekulare Biologie Heidelberg, Germany.
burghaus@mail.uni-marburg.de

Related Resources

Synthetic chimeric DNA constructs with a reduced A + T content coding for full-length merozoite surface protein-1 of *Plasmodium falciparum* (MSP1) and three fragments thereof were expressed in HeLa cells. To target the recombinant proteins to the surface of the host cell the DNA sequences coding for the N-terminal signal sequence and for the putative C-terminal recognition/attachment signal for the glycosyl-phosphatidyl-inositol (GPI)-anchor of MSP1 were replaced by the respective DNA sequences of the human decay-accelerating-factor (DAF). The full-length recombinant protein, hu-MSP1-DAF, was stably expressed and recognised by monoclonal antibodies that bind to the N-terminus or the C-terminus of the native protein, respectively. Its apparent molecular mass is higher as compared to the native protein and it is post-translationally modified by attachment of N-glycans whereas native MSP1 is not glycosylated. Immunofluorescence images of intact cells show a clear surface staining. After permeabilization hu-MSP1-DAF can be detected in the cytosol as well. As judged by protease treatment of intact cells 25% of recombinant MSP1 is located on the surface. This fraction of hu-MSP1-DAF can be cleaved off the cell membrane by phosphatidylinositol-specific phospholipase C indicating that the protein is indeed bound to the cell membrane via a GPI-anchor. Human erythrocytes do not adhere to the surface of mammalian cells expressing either of the constructs made in this study.

PMID: 10593173 [PubMed - indexed for MEDLINE]

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1: Infect Immun. 1994 Jan;62(1):203-9.

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QRI 147 .A35 m/c

Conformationally appropriate expression of the *Toxoplasma* antigen SAG1 (p30) in CHO cells.

Kim K, Bulow R, Kampmeier J, Boothroyd JC.

Department of Microbiology and Immunology, Stanford University School of Medicine, California 94305-5402.

The *Toxoplasma gondii* major surface antigen, called SAG1 or p30, is a highly immunogenic protein which has generated great interest as a diagnostic reagent, as a potential subunit vaccine, and for its role in invasion. Unfortunately, bacterial recombinant protein is grossly misfolded so that, for example, it is not effectively recognized by antibodies to native SAG1. To overcome this, we have turned to expression in CHO cells, using cotransfection of the SAG1 gene and the mouse dihydrofolate reductase (DHFR) gene into CHO cells that are DHFR-. SAG1 expression was amplified by methotrexate coselection of CHO cells in combination with fluorescence-activated cell sorting for SAG1 expression. The resulting population expressed recombinant SAG1 that is recognized by antiserum specific for natural, nonreduced SAG1, indicating that, unlike in bacteria, expression in CHO cells results in proper folding. Processing was at least partially correct in that, like natural SAG1, recombinant SAG1 was attached to the plasma membrane via a glycolipid anchor, although tunicamycin treatment was necessary to prevent N-glycosylation (SAG1 is not glycosylated in the parasite but does have a consensus N-linked site). Finally, purified recombinant SAG1 was recognized by human sera known to be reactive to toxoplasma proteins, indicating that this material has potential as a diagnostic reagent and possibly as a component of a subunit vaccine.

PMID: 8262628 [PubMed - indexed for MEDLINE]

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